MOLECULAR GENETIC ANALYSIS OF ETHAMBUTOL RESISTANCE IN M. TUBERCULOSIS

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The present study of ethambutol resistance in mycobacteria was initiated by cloning and characterizing Mycobacterium avium genes which confer resistance to an otherwise ethambutol-susceptible Mycobacterium smegmatis host. The emb1 ethambutol resistance region is comprised of three noncontiguous segments of approximately 0.4, 8.0, and 1.1 kb (9.5 kb total) within a span of 19 kb. It is not yet known what genes are encoded for by emb1 or what their function is, but one possibility is that emb1 contains genes involved in the biosynthesis of arabinogalactan, a major cell wall component, because this is the presumed site of action of this drug. The genetic complexity of the emb1 region suggests that emb1 may encode for a multienzyme complex that serves as a resistant drug target or that resistance may be due to the overexpression of the sensitive drug target. Further analysis of emb1 is being carried out by DNA sequencing. This work has recently been extended to Mycobacterium tuberculosis, with the immediate goal being to elucidate the genetic basis for ethambutol resistance in multi-drug resistant (MDR) strains. Genomic libraries have been constructed with DNA from an ethambutol resistant derivative of M. tuberculosis strain H37Rv as well as strain CSU24, an MDR strain which is resistant to ethambutol, isoniazid, streptomycin, rifabutin and capreomycin. Both libraries were subjected to a direct selection procedure in M. smegmatis whereby the transformants were selected for their ability to grow on medium containing ethambutol. The H37Rv library was also screened by DNA hybridization using a probe specific to emb1 of M. avium, and the positive cosmid clones were electroporated into M. smegmatis to assay for ethambutol resistance. These methods have yielded three overlapping clones from M. tuberculosis that encode for ethambutol resistance and six overlapping clones that do not express resistance. The putative M. tuberculosis homolog has thus far been localized to a region less than or equal to 12-15 kb, which is considerably shorter than the 19 kb required for emb1, Analysis of the M. tuberculosis ethambutol resistance gene(s) by Southern hybridization and transposon mutagenesis will aid in determining whether the genetic basis for ethambutol resistance is the same in M. tuberculosis and M. avium.

Source: https://www.industrydocuments.ucsf.edu/docs/ggmv0000